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CONTROLLING HUMAN IMMUNODEFICIENCY VIRUS INFECTIONS

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13. ABSTRACT We have begun a series of experiments to examine the growth characteristics of HIV-1 infected T cells in vitro to assist us in identifying factors that may correspond to highly virulent strains of HIV-1 in vivo. In addition, certain in vitro growth characteristics may also correspond to less virulent forms of HIV-1. For example, studies by others have suggested that HIV-1 isolates which readily form syncytia in vitro may be associated with a more rapid rate of disease progression in vivo. Using fluorescent intracellular vital stains, we have expanded upon these studies by examining SupT1 cells for changes in cellular makeup following infection with HIV-1. At present we are staining cells with probes for F-actin, microtubules, p24, and DNA. Our initial studies have indicated that 48 hrs after infection with HIV-1, syncytia no longer express CD4. Using FITC conjugated Leu 3a, we have observed that single cells stain intensely for CD4, while syncytia appear to be CD4 negative. We also observed that syncytia organize their nuclei into a blastula-like formation. In addition, we have observed the extension of pseudopods from the plasma membrane which extend several cell diameters in distance. We are currently examining what role these pseudopods may play in cell recruitment and HIV-1 transmission.			
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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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1. Progress Report

A. In vitro growth characteristics of HIV-1 infected T cells.

1. Fluorescent staining.

We have begun a series of experiments to examine the growth characteristics of HIV-1 infected T cells in vitro to assist us in identifying factors that may correspond to highly virulent strains of HIV-1 in vivo. In addition, certain in vitro growth characteristics may also correspond to less virulent forms of HIV-1. For example, studies by others have suggested that HIV-1 isolates which readily form syncytia in vitro may be associated with a more rapid rate of disease progression in vivo. Using fluorescent intracellular vital stains, we have expanded upon these studies by examining SupT1 cells for changes in cellular makeup following infection with HIV-1. At present we are staining cells with probes for F-actin, microtubules, p24, and DNA. Our initial studies have indicated that 48 hrs after infection with HIV-1, syncytia no longer express CD4. Using FITC conjugated Leu 3a, we have observed that single cells stain intensely for CD4, while syncytia appear to be CD4 negative. We also observed that syncytia organize their nuclei into a blastula-like formation. In addition, we have observed the extension of pseudopods from the plasma membrane which extend several cell diameters in distance. We are currently examining what role these pseudopods may play in cell recruitment and HIV-1 transmission.

2. Examination of cell interactions over prolonged periods of time by videotape.

The physical interaction of cells is difficult to examine visually, since these interactions sometimes extend over long periods of time. Thus, cell movements and interactions that last several hours in duration may be misinterpreted if examined visually for only several minutes. We have demonstrated that these complex interactions are best interpreted if videotaped and then examined during high speed playback. Our initial experiments utilized a video camera mounted to an inverted microscope with a hot air portable incubator attached. A flask containing HIV-1 infected SupT1 cells was then videotaped utilizing a video cassette recorder (VCR). These initial experiments examined cell interactions over periods of up to 30 minutes. We observed, in flasks containing HIV-1 infected SupT1 cells, the directed movement of single cells towards syncytia (Sylwester, et. al., 1993, in press). These results suggest that chemotactic factors may be released from syncytia that result in the recruitment of single cells. This mechanism of cell recruitment by syncytia may play a role in the transmission of HIV-1 to uninfected cells in vitro and possibly in vivo.

Following these initial studies, we decided to examine HIV-1 infected cell cultures continuously over a period of several days. This was accomplished by placing the inverted microscope inside an incubator. A flask containing HIV-1 infected SupT1 cells could then be observed under conditions of constant temperature and CO₂ concentration. The video camera and VCR were again attached as described above. Analysis of videotapes spanning 72 continuous hours demonstrated a wide variety of cell interactions. These included the directed movement of syncytia towards either single cells or other syncytia. Syncytial movement was apparently the result of pseudopod extensions from the forward area of the syncytia. These pseudopods appeared to grasp the flask and pull the syncytia in a directed movement. In addition, we observed that

individual syncytia could migrate towards each other and eventually fuse, forming a single larger syncytial mass. The death of these syncytia was also observed, resulting from the rupture of the plasma membrane and the spilling of cytoplasmic contents. We are presently analyzing a number of the cell motility parameters both single cells and syncytia utilizing these videotapes and a 2-dimensional dynamic image analysis system (2-D-DIAS).

B. Examination of antibody fine specificity to gp160 epitopes in sera from infected individuals from Argentina.

The recent identification of at least five families of HIV-1 throughout the world has increased the need to characterize viral isolates from a variety of geographic regions. These studies are necessary to better understand which formulations of potential HIV-1 vaccines and therapeutics may have the greatest chance of being effective on a worldwide basis. Serologic studies of HIV-1 infected individuals have frequently been conducted in the United States and Europe while other areas have received less attention. For example, Central and South American countries have had few published studies concerning HIV-1 infections. We examined sera from HIV-1 infected individuals from Argentina (n=63) for antibody reactivity to a panel of V3 based synthetic peptides. Our results indicate that the HIV-1 isolates MN, SP2, and NY5 (or phenotypically similar ones) are commonly found in Argentina. HIV-1 isolates LAI and RF appear to be less frequently found among the Argentinean population. In addition, we observed that a lower percentage of sera from infected individuals from Argentina contained antibodies reactive with the gp160 epitope 600-611 than sera from the United States (67% and 94%, respectively). To our knowledge, this represents the first detailed study involving the prevalence of particular HIV-1 isolates in South America. Determining the prevalence of HIV-1 isolates is particularly important for the design of potential HIV-1 vaccines and possible immunotherapeutics.

C. Correlation between sera containing antibodies which recognize HIV-1 V3 based peptides in infected humans and neutralizing activity in vitro.

Since the V3 region of gp120 has been designated the principal neutralizing determinant (PND) of HIV-1, determining the importance of antibodies to this epitope in human sera is critical. This is particularly true, since antibody recognition of this epitope is frequently measured following immunization with potential HIV-1 vaccines. We examined sera from HIV-1 infected individuals from the United States (n=37) and Tanzania (n=74) for antibody reactivity to a panel of V3-based synthetic peptides. We observed that the majority of sera from both countries were reactive against the MN based peptide (84% and 87%, respectively). This data suggests that the MN isolate, or a phenotypically similar one, is commonly found in both countries. When sera were examined for neutralizing activity, a smaller percentage of sera from both countries neutralized the MN isolate in vitro (16% and 47%, respectively). In addition, we were unable to establish a correlation between anti-V3 antibody titers and neutralization titers. We were therefore unable to establish a correlation between the presence of anti-V3 antibodies in human sera and neutralizing activity in vitro (Warren, et. al., J. Virol. 66:5210, 1992). These results suggest that neutralizing epitopes outside of the PND appear to be important in the neutralization of HIV-1 in vitro.

D. Examination of anti-V3 antibodies in various mouse strains receiving multiple immunizations with recombinant gp160.

We examined the immunogenicity of a baculovirus produced gp160 in a variety of mouse strains. We were particularly interested in whether group specific neutralizing antibodies could be produced in these animals following repeated immunizations. Sera from the rgp160 immunized mice (BALB/c, A/J, C57BL, CBA, DBA, and SJL) were initially screened for antibody reactivity to a panel of V3-based synthetic peptides. Of the six strains of mice examined, only the A/J mice produced antibodies which cross-reacted with each of the V3-based synthetic peptides. Sera from the other five strains contained antibodies which remained type specific for the V3 epitope (Wolf, et. al., *Mol. Immunol.* 29:989, 1992). These sera were then examined for their ability to neutralize HIV-1 IIIB and MN in vitro. We were unable to detect neutralizing activity of any sera when measured by reduction in p24 levels (Warren, et. al., *Exp. Clin. Immunogenet.* 26:630, 1993). These results suggest that a baculovirus produced rgp160 may have limited ability to induce group specific neutralizing antibodies in animals following repeated immunization.

E. Comparison of the humoral immune response to HIV-1 gp160 epitopes in sera from infected individuals from the United States and Tanzania.

Regional isolates of HIV-1 have been reported throughout the world. Due to the amino acid sequence variability in the envelope glycoprotein, these regional isolates may potentially differ in their levels of virulence *in vivo*. We compared sera from HIV-1 infected individuals from Tanzania and the United States for anti-gp160 antibody fine specificity. Sera from each country was reacted against a panel of gp160 based synthetic peptides. Several differences in antibody reactivity was observed between sera from these two geographically distinct populations. The greatest difference was observed in antibody reactivity to the 600-611 epitope of gp160. While greater than 90% of the sera from the United States were reactive with this epitope, fewer than 50% of the Tanzanian sera were reactive (Nkya, et. al., *J. Med. Virol.* 37:61, 1992). Significant differences in seroreactivity were also observed against two other gp160 epitopes. These observations suggest that regional isolates of HIV-1 may exist in Tanzania which differ from those commonly found in the United States. This may be particularly important since potential AIDS vaccines which are comprised of common HIV-1 isolates from the United States may not be as effective against African isolates (Warren, et. al., *J. Clin. Micro.* 30:126, 1992).

F. Isolation of HIV-1 from infected individuals from Tanzania.

The isolation and characterization of HIV-1 from infected individuals in Africa could be important for better understanding the epidemiologic differences observed between HIV infections in Africa and the United States. We have isolated HIV-1 from the peripheral blood lymphocytes of two infected individuals from Tanzania (designated NKYA-1 and NKYA-2). Sera from these two individuals were subsequently screen by ELISA against a panel of gp160 based synthetic peptides. We observed that sera from NKYA-1 was unreactive against V3 peptides corresponding to HIV-1 isolates MN and IIIB. In addition, this sera was also unreactive against a peptide corresponding to amino acids 600-611, an epitope which is highly conserved among United States and European isolates. In contrast, sera from NKYA-2 contained antibodies reactive against

peptides corresponding to the V3 region of MN but not IIIB. This sera was also reactive against the 600-611 peptide. These two isolates also had different in vitro growth characteristics. We observed that NKYA-1 isolate formed few syncytia and was not cytotoxic to SupT1 cells. In contrast, NKYA-2 formed many large syncytia and lead to the death of SupT1 cells in culture. These HIV-1 isolates from Tanzania will be useful reagents in testing sera for neutralizing activity in vitro.

G. Longitudinal analysis of the humoral immune response to HIV-1 gp160.

Antibody responses to conserved HIV-1_{IIIB} gp160 epitopes was longitudinally examined in HIV-1 infected individuals. Twelve hundred individuals were evaluated and sequential sera from 25 rapidly progressing (RP) and 30 nonprogressing (NP) patients collected over an average of 4 years were examined. Initial sera from the RP group contained greater reactivity to a gp120 epitope defined by peptide 503-528 than the NP counterparts ($P < .001$). Reactivity declined with sequential serum for the RP group, paralleling disease progression. Conversely, antibody recognition to this site developed in 23% of the NP group with time. However, 60% of the NP group never developed a response to this epitope. This suggests sequential examination of antibody response to an epitope within the gp120 carboxyl-terminus may have prognostic significance. No association between antibodies directed against the gp160 epitopes and in vitro neutralizing activity utilizing the IIIB laboratory isolate was observed.

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